PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
C07D 403/12, 243/14
A61K 31/55, C07D 521/00

A1

(11) International Publication Number: WO 93/17011

(43) International Publication Date: 2 September 1993 (02.09.93)

(21) International Application Number: PCT/GB93/00299

(22) International Filing Date: 12 February 1993 (12.02.93)

(30) Priority data:
9203790.2 21 February 1992 (21.02.92) GB

(71) Applicant *(for all designated States except US)*: MERCK SHARP & DOHME LIMITED [GB/GB]; Hertford Road, Hoddesdon, Hertfordshire EN11 9BU (GB).

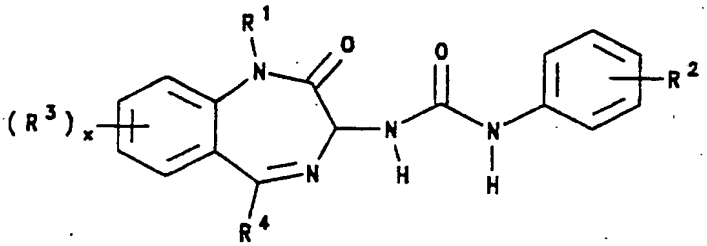
(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : CHAMBERS, Mark, Stuart [GB/GB]; 10 Park Avenue Maisonettes, North Bushey, Watford, Hertfordshire WD2 2BR (GB). FLETCHER, Stephen, Robert [GB/GB]; 10 Clipped Hedge, Hatfield Heath, Nr. Bishops Stortford, Hertfordshire CM22 7EG (GB). MATASSA, Victor, Giulio [GB/GB]; The Duck Street Barns, Furneux Pelham, Hertfordshire SG9 0LA (GB).

(74) Agent: QUILLIN, Helen, Kaye; Merck & Co., Inc., European Patent Department, Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR (GB).

(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: BENZODIAZEPINE DERIVATIVES, AS CCK AND GASTRIN ANTAGONISTS



(I)

(57) Abstract

Compounds of formula (I), and salts and prodrugs thereof wherein: R^1 is H, certain optionally substituted C_{1-6}alkyl, or C_{3-7}cycloalkyl; R^2 is $(CH_2)_q$-tetrazolyl optionally substituted in the tetrazole ring by C_{1-4}alkyl, $(CH_2)_q$-imidazolyl (where q is 0, 1, 2 or 3), $CONHSO_2R^9$, SO_2NHCOR^9 (where R^9 is C_{1-6}alkyl, optionally substituted aryl or trifluoromethyl), SO_2NHR^{10} (where R^{10} is a nitrogen containing heterocycle), cyclopropyl or $(CH_2)_nCO_2H$, where n is 1 or 2; R^3 is C_{1-6}alkyl, halo or NR^6R^7; R^4 is C_{1-7} straight or branched chain alkyl; and x is 0, 1, 2 or 3; are CCK and/or gastrin receptor antagonists. They and compositions thereof are useful in therapy.

AM2

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	MR	Mauritania
AU	Australia	GA	Gabon	MW	Malawi
BB	Barbados	GB	United Kingdom.	NL	Netherlands
BE	Belgium	GN	Guinea	NO	Norway
BF	Burkina Faso	GR	Greece	NZ	New Zealand
BG	Bulgaria	HU	Hungary	PL	Poland
BJ	Benin	IE	Ireland	PT	Portugal
BR	Brazil	IT	Italy	RO	Romania
CA	Canada	JP	Japan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo			SE	Sweden
CH	Switzerland	KR	Republic of Korea	SK	Slovak Republic
CI	Côte d'Ivoire	KZ	Kazakhstan	SN	Senegal
CM	Cameroon	LI	Liechtenstein	SU	Soviet Union
CS	Czechoslovakia	LK	Sri Lanka	TD	Chad
CZ	Czech Republic	LU	Luxembourg	TG	Togo
DE	Germany	MC	Monaco	UA	Ukraine
DK	Denmark	MG	Madagascar	US	United States of America
ES	Spain	ML	Mali	VN	Viet Nam
FI	Finland	MN	Mongolia		

BENZODIAZEPINE DERIVATIVES, AS CCK AND GASTRIN ANTAGONISTS

This invention relates to benzodiazepine compounds which are useful as antagonists of cholecystokinin and gastrin receptors.

Cholecystokinins (CCK) and gastrin are structurally related peptides which exist in gastrointestinal tissue and in the central nervous system (see, V. Mutt, Gastrointestinal Hormones, G.B.J. Green, Ed., Raven Press, N.Y., p.169 and G. Ingression, ibid. p.127).

Cholecystokinins include CCK-33, a neuropeptide of thirty-three amino acids in its originally isolated form (see, Mutt and Jorpes, Biochem. J. 125, 678 (1971)), its carboxylterminal octapeptide, CCK-8 (also a naturally-occurring neuropeptide and the minimum fully active sequence), and 39- and 12-amino acid forms. Gastrin occurs in 34-, 17- and 14-amino acid forms, with the minimum active sequence being the C-terminal tetrapeptide, Trp-Met-Asp-Phe-NH_2, which is the common structural element shared by both CCK and gastrin.

CCKs are believed to be physiological satiety hormones, thereby possibly playing an important role in appetite regulation (G. P. Smith, Eating and Its Disorders, A. J. Stunkard and E. Stellar, Eds, Raven Press, New York, 1984, p. 67), as well as stimulating colonic motility, gall bladder contraction, pancreatic enzyme secretion and inhibiting gastric emptying. They reportedly co-exist with dopamine in certain mid-brain neurons and thus may also play a role in the functioning of dopaminergic systems in the brain, in addition to serving as neurotransmitters in their own right (see A. J. Prange et al., "Peptides in the Central Nervous

System", Ann. Repts. Med. Chem 17, 31, 33 [1982] and references cited therein; J. A. Williams, Biomed Res. 3 107 [1982]; and J.E. Morley, Life Sci. 30, 479 [1982]).

The primary role of gastrin, on the other hand, appears to be stimulation of the secretion of water and electrolytes from the stomach and, as such, is involved in control of gastric acid and pepsin secretion. Other physiological effects of gastrin then include increased mucosal blood flow and increased antral motility. Rat studies have shown that gastrin has a positive trophic effect on the gastric mucosa, as evidenced by increased DNA, RNA and protein synthesis.

There are at least two subtypes of cholecystokinin receptors termed CCK-A and CCK-B (T.H. Moran et al., "Two brain cholecystokinin receptors: implications for behavioural actions", Brain Res., 362, 175-79 [1986]). Both subtypes are found both in the periphery and in the central nervous system.

CCK and gastrin receptor antagonists have been disclosed for preventing and treating CCK-related and/or gastrin related disorders of the gastrointestinal (GI) and central nervous (CNS) systems of animals, especially mammals, and more especially those of humans. Just as there is some overlap in the biological activities of CCK and gastrin, antagonists also tend to have affinity for both CCK-B receptors and gastrin receptors. Other antagonists have activity at the CCK-A subtype.

Selective CCK antagonists are themselves useful in treating CCK-related disorders of appetite regulatory systems of animals as well as in potentiating and prolonging opiate-mediated analgesia [see P. L. Faris et al., Science 226, 1215 (1984)], thus having utility in the treatment of pain. CCK-B and CCK-A antagonists have also been shown to have a direct analgesic effect [M.F.

O'Neill et al., Brain Research, 534 287 (1990)]. Selective CCK and gastrin antagonists are useful in the modulation of behaviour mediated by dopaminergic and serotonergic neuronal systems and thus have utility in the treatment of schizophrenia and depression (Rasmussen et. al., 1991, Eur. J. Pharmacol., 209, 135-138; Woodruff et. al., 1991, Neuropeptides, 19, 45-46; Cervo et. al., 1988, Eur. J. Pharmacol., 158, 53-59), as a palliative for gastrointestinal neoplasms, and in the treatment and prevention of gastrin-related disorders of the gastrointestinal system in humans and animals, such as peptic ulcers, Zollinger-Ellison syndrome, antral G cell hyperplasia and other conditions in which reduced gastrin activity is of therapeutic value, see e.g. U.S. Patent 4,820,834. Certain CCK antagonists are useful anxiolytic agents and can be used in the treatment of panic and anxiety disorders.

CCK has been reported to evoke the release of stress hormones such as adrenocorticotrophic hormone, β-endorphin, vasopressin and oxytocin, CCK may function as a mediator of responses to stress and as part of the arousal system. CCK-A receptors are now known to be present in a number of areas of the CNS and may be involved in modulating all of the above.

CCK may be involved in the regulation of stress and its relationship with drug abuse e.g. alleviation of the benzodiazepine withdrawal syndrome (Singh et. al., 1992, Br. J. Pharmacol., 105, 8-10) and neuroadaptive processes.

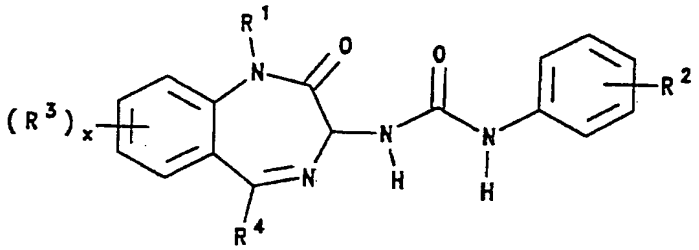
Since CCK and gastrin also have trophic effects on certain tumours [K. Okyama, Hokkaido J. Med. Sci., 206-216 (1985)], antagonists of CCK and gastrin are useful in treating these tumours [see, R.D. Beauchamp et al., Ann. Surg., 202, 203 (1985)].

In the light of discussion in C. Xu et al., Peptides, 8, 1987, 769-772, CCK antagonists may also be effective in neuroprotection.

CCK receptor antagonists have been found to inhibit the contractile effects of CCK on iris sphincter and ciliary muscles of monkey and human eyes (Eur. J. Pharmacol., 211(2), 183-187; A. Bill et al., Acta Physiol. Scand., 138, 479-485 [1990]), thus having utility in inducing miosis for therapeutic purposes.

European patent application no. 0 167 919 discloses benzodiazepine CCK and gastrin antagonists substituted in the 3-position by, inter alia, a phenyl urea and at the 5-position by, inter alia, a C_{1-4}alkyl group. There is no suggestion of the phenyl urea substitution of the compounds of the present invention.

The present invention provides benzodiazepine compounds of formula (I)



(I)

wherein:

R^1 represents H, C_{1-6}alkyl, C_{3-7}cycloalkyl, cyclopropylmethyl, $CH_2CO_2R^5$ (where R^5 is C_{1-4}alkyl) or a group $CH_2CONR^6R^7$ (where R^6 and R^7 each independently represent H or C_{1-4}alkyl, or R^6 and R^7 together form a chain $(CH_2)_p$ where p is 4 or 5);

R^2 represents $(CH_2)_q$-tetrazolyl optionally substituted in the tetrazole ring by C_{1-4}alkyl, $(CH_2)_q$-imidazolyl (where q is 0, 1, 2 or 3), $CONHSO_2R^9$, SO_2NHCOR^9 (where R^9 is C_{1-6}alkyl, optionally substituted aryl or trifluoromethyl), SO_2NHR^{10} (where R^{10} is a nitrogen containing heterocycle), cyclopropyl or $(CH_2)_nCO_2H$, where n is 1 or 2;

R^3 represents C_{1-6}alkyl, halo or $NR^{16}R^{17}$, where R^{16} and R^{17} each independently represent H or C_{1-4}alkyl, or R^{16} and R^{17} together form a chain $(CH_2)_r$ where r is 4 or 5;

R^4 represents C_{1-7} straight or branched chain alkyl;

x is 0, 1, 2 or 3;

and salts and prodrugs thereof.

It will be appreciated that formula (I) is intended to embrace all possible isomers, including optical isomers, and mixtures thereof, including racemates.

The present invention includes within its scope prodrugs of the compounds of formula (I) above. In general, such prodrugs will be functional derivatives of the compounds of formula (I) which are readily convertible in vivo into the required compound of formula (I). Conventional procedures for the selection and preparation of suitable prodrug derivatives are described, for example, in "Design of Prodrugs", ed. H. Bungaard, Elsevier, 1985.

As used herein, unless otherwise stated, alkyl means linear or branched chain alkyl. Examples of suitable alkyl groups include methyl, ethyl, isopropyl and isobutyl groups.

When R^1 represents cycloalkyl, examples of suitable cycloalkyl groups include cyclopropyl,

cyclopentyl and cyclohexyl groups, preferably cyclopropyl.

Halo includes fluoro, chloro, bromo and iodo. Preferably halo will be fluoro or chloro.

Unless otherwise stated, aryl means optionally substituted carbocyclic or heterocyclic aromatic groups, especially phenyl.

Heteroaryl means aromatic rings preferably having 5 or 6 ring atoms and containing at least one atom selected from O, S and a group NR^{13}, where R^{13} is H or C_{1-4} alkyl.

When R^9 is optionally substituted aryl, this will preferably be optionally substituted phenyl. Suitable substituents include C_{1-4}alkyl, C_{1-4}alkoxy, halo and trifluoromethyl. Preferred are compounds wherein R^9 is unsubstituted phenyl or phenyl substituted by C_{1-4}alkyl, for example, phenyl substituted by C_{1-4}alkyl, such as methyl, in the ortho position.

When R^9 is C_{1-6}alkyl, it will preferably represent C_{1-4}alkyl. Particularly preferred are methyl and iso-propyl.

When R^2 is SO_2NHR^{10}, suitable values of R^{10} include, for example, thiazole, thiadiazole and pyrazine.

Preferably R^1 is C_{1-6}alkyl, such as methyl, n-propyl or isobutyl.

Preferably R^2 is in the 3- or 4-position, more preferably the 3-position.

In one preferred group of compounds of formula (I), R^2 is tetrazolyl, more preferably 3-tetrazol-5-yl.

In a further preferred group of compounds of formula (I), R^2 is $CONHSO_2R^9$ or SO_2NHCOR^9, more preferably $CONHSO_2R^9$.

Suitable values for R^9 include methyl, ethyl, i-propyl, t-butyl, phenyl, o-tolyl and trifluoromethyl.

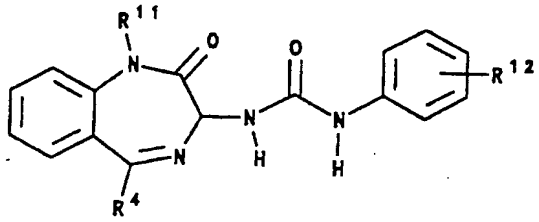
Suitable values for R^3 include methyl and dimethylamino.

Preferably x is 0 or 1, more preferably 0.

Suitably R^4 represents C_{1-4}alkyl, such as methyl, ethyl, i-propyl or t-butyl, preferably i-propyl or t-butyl.

One subgroup of compounds according to the invention is represented by compounds of formula (I) wherein R^1 represents C_{1-6}alkyl, C_{3-7}cycloalkyl, cyclopropylmethyl, $CH_2CO_2R^5$ or $CH_2CONR^6R^7$; R^2 represents $(CH_2)_q$-tetrazolyl wherein one of the N atoms is optionally substituted by methyl, $(CH_2)_q$-imidazolyl, $CONHSO_2R^9$, SO_2NHCOR^9, SO_2NHR^{10}, cyclopropyl or $(CH_2)_nCO_2H$; R^3 is C_{1-6}alkyl or halo; and x is 0 or 1.

A preferred subgroup of compounds according to the invention is represented by compounds of formula (IA), and salts and prodrugs thereof:



(IA)

wherein R^{11} is C_{1-6}alkyl, preferably C_{1-4}alkyl;

R^{12} is tetrazolyl, $CONHSO_2R^9$ or SO_2NHCOR^9, where R^9 is as previously defined, preferably tetrazolyl or $CONHSO_2R^9$; and

R^4 is as defined for formula (I), preferably C_{1-4}alkyl.

Preferred are compounds of formula (IA) wherein R^{12} is in the 3-position of the phenyl ring.

Preferably the salts of the compounds of formula (I) are pharmaceutically acceptable, but non-pharmaceutically acceptable salts may be useful for the preparation of pharmaceutically acceptable salts, and are within the scope of the present invention. The pharmaceutically acceptable salts of the compounds of formula (I) include the conventional non-toxic salts or the quaternary ammonium salts of the compounds of formula (I). For example, such conventional non-toxic salts include basic salts, e.g. sodium and potassium salts.

The salts of the present invention can be synthesized from the compound of formula (I) which contain an acidic moiety by conventional chemical methods. Generally, the salts are prepared by reacting a compound of formula (I) with stoichiometric amounts or with an excess of the desired salt-forming inorganic or organic base in a suitable solvent or combination of solvents.

The present invention also encompasses a pharmaceutical composition comprising a compound of formula (I), or a salt or prodrug thereof, and a pharmaceutically acceptable carrier.

The compounds of formula (I) and their salts and prodrugs may be administered to animals, preferably to mammals, and most especially to a human subject either alone or, preferably, in combination with pharmaceutically acceptable carriers, optionally with known adjuvants, such as alum, in a pharmaceutical compostion, according to standard pharmaceutical practice. The compounds can be administered orally, parenterally, including by intravenous, intramuscular, intraperitoneal or subcutaneous administration, or topically.

For oral use the selected compounds according to this invention may be administered, for example, in the form of tablets or capsules, or as an aqueous solution or suspension. In the case of tablets for oral use, carriers which are commonly used include lactose and corn starch, and lubricating agents, such as magnesium stearate, are commonly added. For oral administration in capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening and/or flavouring agents may be added.

For intramuscular, intraperitoneal, subcutaneous and intravenous use, sterile solutions of the active ingredient are usually prepared, and the pH of the solutions should be suitably adjusted and buffered. For intravenous use, the total concentration of solutes should be controlled in order to render the preparation isotonic.

For topical administration, a compound of formula (I) may be formulated as, for example, a suspension, lotion, cream or ointment.

For topical administration, pharmaceutically acceptable carriers are, for example, water, mixtures of water and water-miscible solvents such as lower alkanols or arylalkanols, vegetable oils, polyalkylene glycols, petroleum based jelly, ethyl cellulose, ethyl oleate, carboxymethylcellulose, polyvinylpyrrolidone, isopropyl myristate and other conventionally-employed non-toxic, pharmaceutically acceptable organic and inorganic carriers. The pharmaceutical preparation may also contain non-toxic auxiliary substances such as emulsifying, preserving, wetting agents, bodying agents and the like, as for example, polyethylene glycols 200,

300, 400 and 600, carbowaxes 1,000, 1,500, 4,000, 6,000 and 10,000, antibacterial components such as quaternary ammonium compounds, phenylmercuric salts known to have cold sterilizing properties and which are non-injurious in use, thimerosal, methyl and propyl paraben, benzyl alcohol, phenyl ethanol, buffering ingredients such as sodium chloride, sodium borate, sodium acetates, gluconate buffers, and other conventional ingredients such as sorbitan monolaurate, triethanolamine, oleate, polyoxyethylene sorbitan monopalmitylate, dioctyl sodium sulfosuccinate, monothioglycerol, thiosorbitol, ethylenediamine tetraacetic acid, and the like.

The compounds of formula (I) antagonise CCK and/or gastrin and are useful for the treatment and prevention of disorders including central nervous system disorders wherein CCK and/or gastrin may be involved. Examples of such disease states include gastrointestinal diseases, including gastrointestinal ulcers, such as peptic and duodenal ulcers, irritable bowel syndrome, gastroesophagenal reflux disease or excess pancreatic or gastrin secretion, acute pancreatitis, or motility disorders; central nervous system disorders, including central nervous system disorders caused by CCK interaction with dopamine, serotonin and other monoamine neurotransmitters, such as neuroleptic disorders, tardive dyskinesia, Parkinson's disease, psychosis or Gilles de la Tourette syndrome; depression, such as depression resulting from organic disease, secondary to stress associated with personal loss or idiopathic depression; schizophrenia; disorders of appetite regulatory systems; Zollinger-Ellison syndrome, antral and cell hyperplasia, or pain.

The compounds of formula (I) are particularly useful in the treatment or prevention of neurological

disorders involving anxiety disorders and panic disorders, wherein CCK and/or gastrin is involved. Examples of such disorders include panic disorders, anxiety disorders, panic syndrome, anticipatory anxiety, phobic anxiety, panic anxiety, chronic anxiety and endogenous anxiety.

The compounds of formula (I) are also useful for directly inducing analgesia, opiate or non-opiate mediated, as well as anaesthesia or loss of the sensation of pain.

The compounds of formula (I) may further be useful for preventing or treating the withdrawal response produced by chronic treatment or abuse of drugs or alcohol. Such drugs include, but are not limited to benzodiazepines, cocaine, alcohol and nicotine.

The compounds of formula (I) may further by useful in the treatment of stress and its relationship with drug abuse.

The compounds of formula (I) may further be useful in the treatment of oncologic disorders wherein CCK may be involved. Examples of such oncologic disorders include small cell adenocarcinomas and primary tumours of the central nervous system glial and neuronal cells. Examples of such adenocarcinomas and tumours include, but are not limited to, tumours of the lower oesophagus, stomach, intestine, colon and lung, including small cell lung carcinoma.

The compounds of formula (I) may also be useful as neuroprotective agents, for example, in the treatment and/or prevention of neurodegenerative disorders arising as a consequence of such pathological conditions as stroke, hypoglycaemia, cerebral palsy, transient cerebral ischaemic attack, cerebral ischaemia during cardiac pulmonary surgery or cardiac arrest, perinatal asphyxia,

epilepsy, Huntington's chorea, Alzheimer's disease, Amyotrophic Lateral Sclerosis, Parkinson's disease, Olivo-ponto-cerebellar atrophy, anoxia such as from drowning, spinal cord and head injury, and poisoning by neurotoxins, including environmental neurotoxins.

The compounds of formula (I) may further be used to induce miosis for therapeutic purposes after certain types of examination and intraocular surgery. An example of intraocular surgery would include cateract surgery with implantation of an artificial lens. The CCK antagonist compounds of this invention can be used to prevent miosis occuring in association with iritis, ureitis and trauma.

The present invention therefore provides a compound of formula (I) or a salt or prodrug thereof for use in the preparation of a medicament for the treatment of a physiological disorder involving CCK and/or gastrin.

The present invention also provides a compound of formula (I), or a salt or prodrug thereof, for use in therapy.

In a further or alternative embodiment the present invention provides a method for the treatment or prevention of a physiological disorder involving CCK and/or gastrin which method comprises administration to a patient in need thereof of a CCK and/or gastrin antagonising amount of a compound of formula (I).

When a compound according to formula (I) is used as an antagonist of CCK or gastrin in a human subject, the daily dosage will normally be determined by the prescibing physician with the dosage generally varying according to the age, weight, and response of the individual patient, as well as the severity of the patient's symptoms. However, in most instances, an effective daily dosage wll be in the range from about

0.005mg/kg to about 100mg/kg of body weight, and preferably, of from 0.05mg/kg to about 50mg/kg, such as from about 0.5mg/kg to about 20mg/kg of body weight, administered in single or divided doses. In some cases, however, it may be necessary to use dosages outside these limits. For example, animal experiments have indicated that doses as low as 1ng may be effective.

In effective treatment of panic syndrome, panic disorder, anxiety disorder and the like, preferably about 0.05 mg/kg to about 0.5 mg/kg of CCK antagonist may be administered orally (p.o.), administered in single or divided doses per day (b.i.d.). Other routes of administration are also suitable.

For directly inducing analgesia, anaesthesia or loss of pain sensation, the effective dosage preferably ranges from about 100 ng/kg to about 1mg/kg by intravenous administration. Oral administration is an alternative route, as well as others.

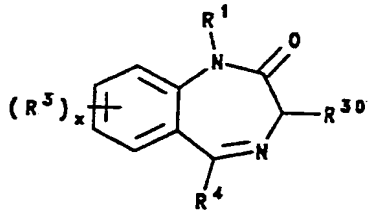
In the treatment or irritable bowel syndrome, preferably about 0.1 to 10 mg/kg of CCK antagonist is administered orally (p.o.), administered in single or divided doses per day (b.i.d.). Other routes of administration are also suitable.

The use of a gastrin antagonist as a tumour palliative for gastrointestinal neoplasma with gastrin receptors, as a modulator of central nervous activity, treatment of Zollinger-Ellison syndrome, or in the treatment of peptic ulcer disease, an effective dosage of preferably about 0.1 to about 10 mg/kg administered one-to-four times daily is indicated.

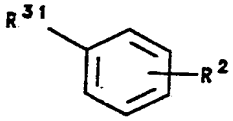
For use as neuroprotective agents the effective dosage preferably ranges from about 0.5mg/kg to about 20mg/kg.

Because these compounds antagonise the function of CCK in animals, they may also be used as feed additives to increase the food intake of animals in daily dosage of preferably about 0.05mg/kg to about 50mg/kg of body weight.

The compounds of formula (I) may be prepared by processes analogous to those described in European patent specification No. 0284256. For example, a compound of formula (I) may be prepared by reaction of an intermediate of formula (II) with a compound of formula (III)



(II)



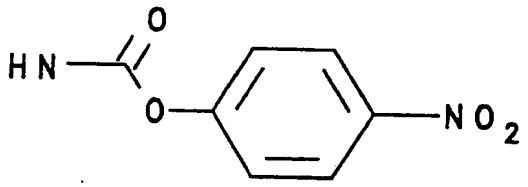
(III)

wherein R^1, R^2, R^3, R^4 and x are as defined for formula (I), one of R^{30} and R^{31} represents NH_2 and the other of R^{30} and R^{31} represents N=C=O or an activated carbamate.

When one of R^{30} and R^{31} represents N=C=O, the reaction is preferably conducted in a suitable organic solvent, such as an ether, for example, tetrahydrofuran, at room temperature.

When one of R^{30} and R^{31} represents an activated carbamate the reaction is effected in the presence of a base. Suitable bases for use in the reaction include tertiary amines, for example, triethylamine. Preferably R^{30} represents an activated carbamate and R^{31} represents NH_2.

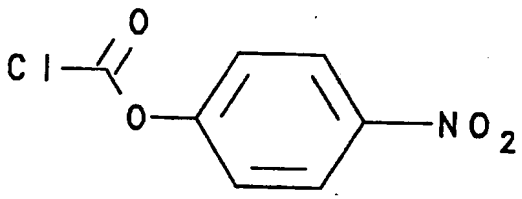
The activated carbamate will suitably be an appropriately substituted aryl carbamate, for example



The reaction is conveniently effected in a suitable organic solvent, for example, dimethylformamide, at ambient or elevated temperature. Preferably the reaction is conducted at approximately 50°C.

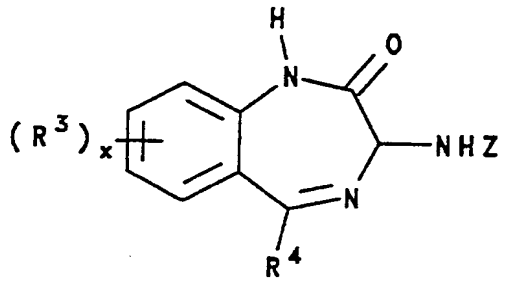
Intermediates of formula (II) wherein R^{30} is N=C=O (IIB) may be prepared from corresponding amines of formula (II) wherein R^{30} is NH_2 (IIA) by conventional methods, for example, by treatment with triphosgene.

Intermediates of formula (II) where R^{30} is an activated carbamate (IIC) may be prepared from compounds of formula (IIA) by reaction with a suitable chloroformate, for example



in the presence of a base, such as a tertiary amine, for example, triethylamine.

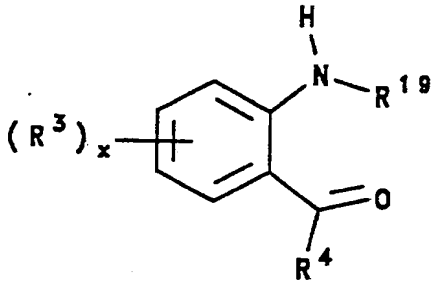
Intermediates of formula (IIA) may be prepared from compounds of formula (VI)



(VI)

wherein R^3, R^4 and x are is as defined for formula (I) and Z is a protecting group; by reaction with a reagent suitable to introduce the group R^1, for example a halide of formula R^1Hal where Hal represents halo such as bromo or iodo, in the presence of a base, such as an alkali metal hydride or an alkaline earth metal carbonate, for example sodium hydride or caesium carbonate; or a suitable dialkyl acetal of dimethyl formamide in a suitable organic solvent, e.g. toluene followed by deprotection.

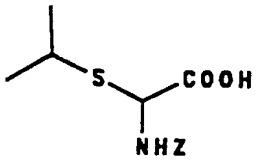
Compounds of formula (VI) may be prepared from compounds of formula (VII)



(VII)

wherein R^3, R^4 and x are is as defined for formula (I) and R^{19} is H, by a reaction sequence comprising:

(i) reaction with a compound of formula (VIII)



(VIII)

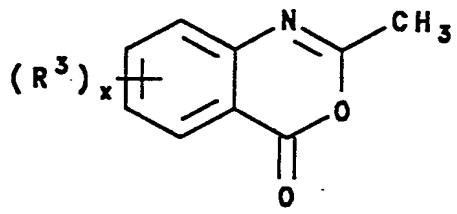
wherein Z is as defined above, in the presence of a base, such as a tertiary amine, for example triethylamine or N-methyl morpholine, and a coupling reagent. Any of the coupling reagents commonly used in peptide synthesis are suitable, for example, 1,3-dicyclohexylcarbodiimide (DCC) or isobutyl chloroformate;

(ii) Treatment with gaseous ammonia, preferably in the presence of a mercury containing catalyst, such as mercury(II) chloride. The reaction is conveniently effected in a suitable organic solvent, such as an ether, for example, tetrahydrofuran;

(iii) Treatment with an organic acid, for example acetic or propionic acid, optionally in the presence of an ammonium salt, for example ammonium acetate.

Compounds of formula (VII) wherein R^{19} is H may be prepared from corresponding compounds of formula (VII) wherein R^{19} is $COCH_3$ by treatment with a mineral acid, for example hydrochloric acid, or base hydrolysis, for example, using aqueous sodium hydroxide. The reaction is conveniently affected in refluxing methanol.

Compounds of formula (VII) wherein R^{19} is $COCH_3$ may be prepared from compounds of formula (IX)

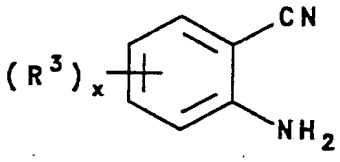


(IX)

wherein R^3 and x defined as for formula (I), by reaction with a Grignard reagent of formula R^4MgHal wherein Hal is halo such as chloro, bromo or iodo.

Compounds of formula (IX) may be prepared by known methods,e.g. see D.A. Walsh, Synthesis, 677, (1980).

Alternatively, compounds of formula (VII) wherein R^{19} is H may be prepared by reaction of a compound of formula (X)



(X)

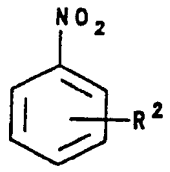
wherein R^3 and x are as previously defined, with a Grignard reagent of formula R^4MgHal wherein R^4 is as previously defined and Hal is halo such as chloro, bromo or iodo.

Compounds of formula (X) are commercially available or may be prepared from commercially available compounds by conventional methods.

Intermediates of formula (III) wherein R^{31} represents N=C=O (IIIB) or an activated carbonate (IIIC) may be prepared from the corresponding amines of formula

(III) wherein R^{31} is NH_2 (IIIA) by conventional methods analogous to those described for the preparation of intermediates of formulae (IIB) and (IIC) from amines of formula (IIA).

Intermediates of formula (IIIA) are known compounds, or may be prepared from the corresponding nitro compounds of formula (XI)



(XI)

wherein R^2 is as defined for formula (I), by reduction.

Suitably the reduction is effected by catalytic hydrogen, for example, using a noble metal catalyst such as palladium which may be supported, e.g. on carbon. The reaction is conveniently effected in a suitable organic solvent, such as an alcohol, e.g. ethanol.

Compounds of formula (XI) are commercially available or may be prepared by conventional procedures which will be readily apparent to one skilled in the art.

Where the above-described process for the preparation of the compounds according to the invention gives rise to mixtures of stereoisomers these isomers may, if desired, be separated, suitably by conventional techniques such as preparative chromatography.

The novel compounds may be prepared in racemic form, or individual enantiomers may be prepared either by enantiospecific synthesis or by resolution. The novel compounds may, for example, be resolved into their component enantiomers by standard techniques, such as the formation of diastereomeric pairs by salt formation with

an optically active acid, such as (-)-di-p-toluoyl-L-tartaric acid and/or (+)-di-p-toluoyl-D-tartaric acid followed by fractional crystallization and regeneration of the free base. The novel compounds may also be resolved by formation of diastereomeric esters or amides, followed by chromatographic separation and removal of the chiral auxiliary. Alternatively, enantiomers of the novel compounds may be separated by HPLC using a chiral column.

During any of the above synthetic sequences it may be necessary and/or desirable to protect sensitive or reactive groups on any of the molecules concerned. This may be achieved by means of conventional protecting groups, such as those described in Protective Groups in Organic Chemistry, ed. J.F.W. McOmie, Plenum Press, 1973; and T.W. Greene and P.M.G. Wutts, Protective Groups in Organic Synthesis, John Wiley & Sons, 1991. The protecting groups may be removed at a convenient subsequent stage using methods known from the art.

The following examples are provided to assist in a further understanding of the invention. Particular materials employed, species and conditions are intended to be further illustrative of the invention and not limitative of the scope thereof.

Chromatography was performed on silica gel.

EXAMPLE 1

N-[3(R,S)-2,3-Dihydro-1,5-dimethyl-2-oxo-1H-1,4-benzodiazepin-3-yl] N'-[3-tetrazol-5-ylphenyl]urea

Step 1: 5-(3-Nitrophenyl)tetrazole

To a solution of 3-cyanonitrobenzene (20g, 0.13mol) in 1-methyl-2-pyrrolidinone (200ml) was added triethylamine hydrochloride (27.9g, 0.20mol) followed by sodium azide (26.4g, 0.40mol). The mixture was heated at 160°C for 1.5h, then cooled to ambient temperature, poured into ice water (100ml) and acidified using 5M HCl. The solid which precipitated from the mixture was filtered, washed with water and dried under vacuum at 50°C to afford the title tetrazole (22.1g, 86%) as a beige powder, mp 154-156°C. ^{1}H NMR (360MHz, $CDCl_3$) δ 7.59 (1H, t, J = 8Hz), 8.19 (1H, d, J = 8Hz), 8.36 (1H, d, J = 8Hz), 8.86 (1H, s).

Step 2: 5-(3-Aminophenyl)tetrazole, hydrochloride salt

To a solution of 5-(3-nitrophenyl)tetrazole (22g, 0.12mol) in ethanol (500ml) was added 10% palladium on carbon (1.5g, 7% ($^w/_w$)) in hydrochloric acid (23ml of a 5M solution). The mixture was hydrogenated at 40 psi for 10 min, then the catalyst filtered off and washed with water. The solvents were evaporated *in vacuo* and the brown solid azeotroped with toluene (4 x 100ml).

The resulting solid was triturated with hot ethanol to give 5-(3-aminophenyl)tetrazole hydrochloride (16.3g, 71%) as a beige powder, mp 203-205°C. ^{1}H NMR (360MHz, D_2O) δ 7.63 (1H, d, J = 9Hz), 7.75 (1H, t, J = 8Hz), 8.00 (2H, m).

Step 3: 1,3-Dihydro-5-methyl-3(R,S)-[(benzyloxycarbonyl)-amino]-2H-1,4-benzodiazepin-2-one

@-(Isopropylthio)-N-(benzyloxycarbonyl)glycine (36g, 0.127mol) was dissolved in dichloromethane (1000ml) and cooled to 0°C. The stirred solution was then treated with N-methylmorpholine (13.9ml, 0.127mol) followed by isobutyl chloroformate (16.5ml, 0.127mol). The resulting reaction mixture was stirred for a further 15 min at 0°C, then heated to reflux. The refluxing reaction mixture was treated dropwise, over 20 min, with a solution of 2-aminoacetophenone (15g, 0.11mol) in dichloromethane (154ml). After addition was complete the reaction was stirred at ambient temperature for 16h. The mixture was then washed in succession with 10% citric acid solution (2 x 50ml), saturated sodium bicarbonate solution (2 x 500ml) and brine (500ml). The dried ($MgSO_4$) organic phase was evaporated to afford the crude product as a yellow oil, which was used without further purification.

The crude (isopropylthio)glycinamide was dissolved in anhydrous tetrahydrofuran (800ml) and cooled to 0°C.

Ammonia gas was bubbled through the stirred solution for 30 min before addition of mercuric chloride (36g, 0.132mol) in one portion. Ammonia was continually bubbled through the solution for a further 2h, then the suspended solids were filtered off. The solvent was evaporated *in vacuo* to leave an oil, which was used without further purification.

The crude @-aminoglycinamide was dissolved in glacial acetic acid (500ml) and treated with ammonium acetate (40g, 0.52mol). The resulting reaction mixture was stirred at room temperature overnight, before removing the solvent *in vacuo*. The residue was partitioned between ethyl acetate (300ml) and 1N sodium hydroxide solution (300ml). The organic phase was separated, dried ($MgSO_4$) and evaporated. The residue was chromatographed on silica, using 1:1 petrol:ethyl acetate as eluent to afford 1,3-dihydro-5-methyl-3(R,S)-[(benzyloxycarbonyl)-amino]-2H-1,4-benzodiazepin-2-one (7.6g, 21%) as a colourless solid. ^1H NMR (360MHz, $CDCl_3$) δ 2.47 (3H, s), 5.05-5.25 (3H, m), 6.50 (1H, d, J = 8Hz), 7.0-7.7 (9H, m), 8.7 (1H, s).

Step 4: 1,3-Dihydro-1,5-dimethyl-3(R,S)-[(benzyloxycarbonyl)-amino]-2H-1,4-benzodiazepin-2-one

A solution of 1,3-dihydro-5-methyl-3(R,S)-[(benzyloxycarbonyl)-amino]-2H-1,4-benzodiazepin-2-one (2g, 6.2mmol) in dimethylformamide (20ml), under an atmosphere of nitrogen, was treated with sodium hydride (0.26g of a 57%

dispersion in mineral oil, 6.2mmol) in one portion, at -10°C. After 30 min, iodomethane (0.39ml, 6.2mmol) was added and the resulting mixture stirred at room temperature for 3h. The solvent was then evaporated and the crude residue partitioned between water (20ml) and dichloromethane (3 x 20ml). The combined organic phase was washed with brine (20ml), dried ($MgSO_4$) and evaporated. The residue was triturated with ether to afford the title compound (1.05g, 50%) as a colourless solid. ^1H NMR (360MHz, $CDCl_3$) δ 2.52 (3H, s), 3.42 (3H, s), 5.0-5.25 (3H, m), 6.65 (1H, d, J = 8Hz), 7.2-7.7 (9H, m).

Step 5: N-[3(R,S)-2,3-Dihydro-1,5-dimethyl-2-oxo-1H-1,4-benzodiazepin-3-yl] N'-[3-tetrazol-5-ylphenyl]urea

1,3-Dihydro-1,5-dimethyl-3(R,S)-[(benzyloxycarbonyl)amino]-2H-1,4-benzodiazepin-2-one (0.6g, 1.78mmol) was dissolved in formic acid/methanol (104ml of a 4.5% (v/v) solution), and added to a stirred suspension of 10% palladium on carbon (0.22g) in formic acid methanol (22ml of a 4.5% (v/v) solution). After 45 min the catalyst was removed by filtration, the filtrate evaporated and the residue partitioned between 10% sodium carbonate solution (20ml) and dichloromethane (3 x 20ml). The combined organic phase was dried (Na_2SO_4) and evaporated to give a clear oil, which was used without further purification.

To a suspension of 5-(3-aminophenyl)tetrazole hydrochloride (0.274g, 1.4mmol) in tetrahydrofuran (10ml) was added triethylamine (0.38ml, 2.8mmol). The mixture was cooled to 0°C, triphosgene (0.13g, 0.43mmol) added and adjusted to pH8 by the addition of triethylamine (0.24ml, 1.76mmol). The ice bath was removed and the mixture stirred at room temperature for 30 min. A solution of the aminobenzodiazepine (0.217g, 1.06mmol), from the above procedure, in tetrahydrofuran (15ml) was added dropwise to the mixture. The reaction mixture was stirred at room temperature for 2h, then diluted with ethyl acetate (20ml) followed by 20% aqueous acetic acid (20ml). After stirring for a further 15 min a colourless precipitate was filtered off. The solid was triturated with ether and then hot methanol to afford the desired material (73mg, 18%) as a colourless solid, mp 208-210°C. ^{1}H NMR (360MHz, D_6-DMSO) δ 2.42 (3H, s), 3.35 (3H, s), 5.15 (1H, d, J = 8Hz), 7.3-7.85 (8H, m), 8.2 (1H, s), 9.25 (1H, s).

EXAMPLE 2

N-[3(R,S)-2,3-Dihydro-1-(2-methylpropyl)-2-oxo-5-(2-propyl)-1H-1,4-benzodiazepin-3-yl] N'-[3-tetrazol-5-ylphenyl]urea

Step 1: 1,3-Dihydro-5-(2-propyl)-3(R,S)-[(benzyloxycarbonyl)-amino]-2H-1,4-benzodiazepin-2-one

@-(Isopropylthio)-N-(benzyloxycarbonyl)glycine (8.7g, 30.7mmol) was dissolved in dichloromethane (130ml) and cooled to 0°C. The stirred solution was then treated with N-methylmorpholine (3.4ml, 30.7mmol) followed by isobutylchloroformate (4.0ml, 30.7mmol). The resulting reaction mixture was stirred for a further 15 min at 0°C, then heated to reflux. The refluxing reaction mixture was treated dropwise, over 20 min, with a solution of 1-(3-aminophenyl)-2-methyl-propan-1-one (5g, 30.7mmol; ref: Synthesis 1991, p 56) in dichloromethane (50ml). After addition was complete the reaction was stirred at reflux for 2 h. The mixture was then washed in succession with 10% citric acid solution (2 x 100ml), saturated sodium bicarbonate solution (2 x 100ml) and brine (100ml). The dried ($MgSO_4$) organic phase was evaporated to afford the crude product as a yellow oil which was used without further purification.

The crude (isopropylthio)glycinamide was dissolved in anhydrous tetrahydrofuran (500ml) and cooled to 0°C. Ammonia gas was bubbled through the stirred solution for 30 min before addition of mercuric chloride (12.5g, 46mmol) in one portion. Ammonia was continually bubbled through the stirred solution for a further 4h, then the suspended solids were filtered off. The solvent was evaporated *in vacuo* to leave an oil, which was used without further purification.

The crude @-aminoglycinamide was dissolved in glacial

acetic acid (200ml) and treated with ammonium acetate (11.1g, 0.14mol). The resulting mixture was stirred at room temperature overnight before removing the solvent *in vacuo*. The residue was partitioned between ethyl acetate (100ml) and 1N sodium hydroxide solution (100ml). The organic phase was separated, dried ($MgSO_4$) and evaporated. The residue was chromatographed on silica using 1:1 petrol:ethyl acetate as eluent to afford 1,3-dihydro-5-(2-propyl)-3(R,S)-[(benzyloxycarbonyl)-amino]-2H-1,4-benzodiazepin-2-one (530mg, 5%) as a colourless solid. ^1H NMR (250MHz, D_6-DMSO) δ 0.90 (6H, d, J = 7Hz), 3.14 (1H, m), 5.10 (3H, m), 6.52 (1H, d, J = 10Hz), 7.0-7.6 (9H, m), 9.50 (1H, brs).

Step 2: 1,3-Dihydro-1-(2-methylpropyl)-5-(2-propyl)-3(R,S)-[(benzyloxycarbonyl)-amino]-2H-1,4-benzodiazepin-2-one

A solution of 1,3-dihydro-5-(2-propyl)-3(R,S)-[(benzyloxycarbonyl)-amino]-2H-1,4-benzodiazepin-2-one (500mg, 1.42mmol) in dimethylformamide (10ml) under an atmosphere of nitrogen, was treated with sodium hydride (57mg of a 57% dispersion in mineral oil, 1.42mmol) in one portion at 0°C. After 1h, 2-methylpropyl iodide (0.17ml, 1.50mmol) was added and the resulting mixture was stirred at room temperature for 16 h. The solvent was then evaporated and the crude residue partitioned between water (15ml) and dichloromethane (2 x 20ml). The combined organic phase was washed with brine (20ml), dried ($MgSO_4$) and evaporated. The

residue was chromatographed on silica using 1:3 ethyl acetate:petrol as eluent, to afford 1,3-dihydro-1-(2-methylpropyl)-5-(2-propyl)-3(R,S)-[(benzyloxycarbonyl)-amino]-2H-1,4-benzodiazepin-2-one (530mg, 92%) as a colourless solid. ^{1}H NMR (250MHz, $CDCl_3$) δ 0.72 (3H, d, J = 8Hz), 0.80 (3H, d, J = 8Hz), 1.02 (3H, d, J = 7Hz), 1.30 (3H, d, J = 7Hz), 1.64 (1H, m), 3.10 (1H, m), 3.42 (1H, dd, J = 15Hz, 5Hz), 4.30 (1H, dd, J = 12.5Hz, J = 7.5Hz), 5.12 (3H, m), 6.55 (1H, d, J = 10Hz), 7.10-7.70 (9H, m).

Step 3: N-[3(R,S)-2,3-Dihydro-1-(2-methylpropyl)-2-oxo-5-(2-propyl)-1H-1,4-benzodiazepin-3-yl] N'-[3-tetrazol-5-ylphenyl]urea

1,3-Dihydro-1-(2-methylpropyl)-5-(2-propyl)-3(R,S)-[(benzyloxycarbonyl)-amino]-2H-1,4-benzodiazepin-2-one (500mg, 1.23mmol) was dissolved in formic acid/methanol (25ml of a 4.5% (v/v) solution), and added to a stirred suspension of 10% palladium on carbon (100mg) in formic acid/methanol (25ml of a 4.5% (v/v) solution). After 30 min the catalyst was removed by filtration, the filtrate evaporated and the residue partitioned between 10% sodium carbonate solution (20ml) and dichloromethane (3 x 20ml). The combined organic phase was dried (Na_2SO_4) and evaporated to give a clear oil which was used without further purification.

To a suspension of 5-(3-aminophenyl)tetrazole hydrochloride

(Example 1, Step 2, 316mg, 1.6mmol) in tetrahydrofuran (10ml) was added triethylamine (0.44ml, 3.2mmol). The mixture was cooled to 0°C, triphosgene (157mg, 0.53mmol) added and adjusted to pH8 by the addition of triethylamine (0.22ml, 1.6mmol). The ice-bath was removed and the mixture was stirred at room temperature for 30 min. A solution of the aminobenzodiazepine (355mg, 1.2mmol), from the above procedure, in tetrahydrofuran (10ml) was added dropwise to the mixture. The reaction mixture was stirred at room temperature for 2h, then diluted with ethyl acetate (20ml) followed by 20% aqueous acetic acid (20ml). The organic phase was separated, dried (Na_2SO_4) and evaporated. Purification was achieved by preparat. HPLC, on a C-18 column with CH_3CN:H_2O:HOAc 50:49:1 as eluent affording the product as a colourless solid.ret. time 10 min, mp = 220°C dec. (80mg, 14%). ^1H NMR (360MHz, D_6-DMSO) δ 0.64 (3H, d, J = 7Hz), 0.76 (3H, d, J = 7Hz), 0.92 (3H, d, J = 8Hz), 1.20 (3H, d, J = 6Hz), 1.49 (1H, m), 3.33 (1H, m), 3.64 (1H, dd, J = 15Hz, 5Hz), 4.16 (1H, dd, J = 12Hz, 7Hz), 5.07 (1H, d, J = 8Hz), 7.20-7.80 (8H, m), 8.13 (1H, s), 9.22 (1H, s).

EXAMPLE 3

N-[3(R,S)-2,3-Dihydro-5-(1,1-dimethylethyl)-1-(2-methylpropyl)-2-oxo-1H-1,4-benzodiazepin-3-yl] N'-(3-(methylsulphonylaminocarbonyl)phenyl]urea

Step 1: 1-(Methylsulphonylaminocarbonyl)-3-nitrobenzene

A solution of methylsulphonamide (5.37g, 57mmol) in anhydrous dichloromethane (100ml), cooled to 0°C was treated with triethylamine (7.9ml, 57mmol) followed by a solution of 3-nitrobenzoyl chloride (10g, 54mmol) in anhydrous dichloromethane (100ml) dropwise. After stirring for 2h at 0°C, the reaction mixture was washed with 1M HCl (100ml). The precipitate which formed was collected by filtration and triturated with diethyl ether and was then recrystallised from hot methanol to afford the title compound (4.3g, 31%) as a colourless crystalline solid, mp 175-178°C. ^{1}H NMR (360MHz, D_6-DMSO) δ 3.42 (3H, s), 7.82 (1H, dd, J = 8.0 and 8.0Hz), 8.38 (1H, d, J = 8.0Hz), 8.49 (1H, d, J = 8.0Hz), 8.80 (1H, s).

Step 2: 1-(Methylsulphonylaminocarbonyl)-3-aminobenzene

To a suspension of 1-(methylsulphonylaminocarbonyl)-3-nitrobenzene (4g, 16mmol) in ethanol (100ml) was added 10% palladium on carbon (0.5g, 12.5% ($^{w}/_{w}$)) in water (5ml). The mixture was hydrogenated at 40 psi for 10 min then the catalyst was filtered off and washed with ethanol. The solvent was evaporated *in vacuo* to give the title compound (2.9g, 83%) as a tan powder after trituration with diethyl ether, mp 153-155°C. ^{1}H NMR (360MHz, D_6-DMSO) δ 3.3 (3H, s), 6.79 (1H, d, J = 7.7Hz), 7.05 (1H, d, J = 7.7Hz), 7.08 (1H, d, J = 1.9Hz), 7.13 (1H, dd, J = 7.7 and 7.7Hz).

Step 3: 1-(2-Aminophenyl)-2,2-dimethylpropan-1-one

To a solution of 1-(2-nitrophenyl)-2,2-dimethylpropan-1-one (5g, 24mmol) (ref: Aus. J. Chem., 34, 1875-8 (1981)) in ethanol (100ml) was added 10% palladium on carbon (0.5g, 10% ($^{w}/_{w}$)) in hydrochloric acid (5ml of a 5M solution). The mixture was hydrogenated at 25 psi for 20 min, then the catalyst was filtered off and washed with methanol. The solvents were evaporated *in vacuo*. The oily residue was partitioned between ethyl acetate (100ml) and saturated sodium bicarbonate solution (50ml). The organic phase was separated, washed with brine (25ml), dried (Na_2SO_4) and evaporated. Trituration of the solid with diethyl ether afforded the title compound as a colourless solid (3g, 70%). ^{1}H NMR (250MHz, $CDCl_3$) δ 1.38 (9H, s), 5.64 (2H, brs), 6.60-7.30 (4H, m).

Step 4: 1,3-Dihydro-5-(1,1-dimethylethyl)-3(R,S)-[(benzyloxycarbonyl)-amino]-2H-1,4-benzodiazepin-2-one

@-(Isopropylthio)-N-(benzyloxycarbonyl)glycine (1.67g, 6.5mmol) was dissolved in dichloromethane (100ml) and cooled to 0°C. The stirred solution was then treated with N-methylmorpholine (0.71ml, 6.5mmol) followed by isobutylchloroformate (0.84ml, 6.5mmol). The resulting reaction mixture was stirred for a further 15 min at 0°C, then heated to reflux. The refluxing reaction mixture was then treated dropwise, over 15 min, with a solution of 1-(2'-aminophenyl)-2,2-

dimethylpropan-1-one (1.1g, 6.2mmol) in dichloromethane (20ml). After addition was complete the reaction was stirred at reflux for 3 h. The mixture was then washed in succession with 1.0N citric acid (2 x 25ml), saturated sodium bicarbonate solution (2 x 25ml) and brine (50ml). The dried ($MgSO_4$) organic phase was evaporated to afford the crude product as a yellow solid, which was used without further purification.

The crude (isopropylthio)glycinamide was dissolved in anhydrous tetrahydrofuran (120ml) and cooled to 0°C. Ammonia gas was bubbled through the stirred solution for 30 min before addition of mercuric chloride (2.5g, 9.3mmol) in one portion. Ammonia was continually bubbled through the stirred solution for 2 hours, then the suspended solids were filtered off. The solvent was evaporated *in vacuo* to leave an oil, which was used without purification.

The crude @-aminoglycinamide was dissolved in glacial acetic acid (100ml) and treated with ammonium acetate (2.3g, 30mmol). The resulting mixture was stirred at room temperature overnight, before removing the solvent *in vacuo*. The residue was partitioned between ethyl acetate (100ml) and 1.0N sodium hydroxide solution (100ml). The organic phase was separated, dried ($MgSO_4$) and evaporated. The residue was chromatographed on silica, using 2:1 petrol:ethyl acetate as eluent, to afford the title compound as a colourless solid (750mg, 33%). ^1H NMR (250MHz, D_6-DMSO) δ 1.00 (9H, s), 4.84-4.86

(1H, m), 5.04 (2H, s), 7.10-7.56 (7H, m), 7.75 (1H, d, J = 8Hz), 8.20 (1H, d, J = 7Hz).

Step 5: -1,3-Dihydro-5-(1,1-dimethylethyl)1-(2-Methylpropyl)-3(R,S)-[(benzyloxycarbonyl)-amino]-2H-1,4-benzodiazepin-2-one

A solution of 1,3-dihydro-5-(1,1-dimethylethyl)-3(R,S)-[(benzyloxycarbonyl)-amino]-2H-1,4-benzodiazepin-2-one (500mg, 1.4mmol) in dimethylformamide (10ml) under an atmosphere of nitrogen, was treated with sodium hydride (54mg of a 57% dispersion in mineral oil, 1.4mmol) in one portion at 0°C. After 1h, 2-methylpropyl iodide (0.17ml, 1.45mmol) was added and the resulting mixture stirred at room temperature for 16 h. The solvent was then evaporated and the crude residue partitioned between water (15ml) and dichloromethane (2 x 20ml). The combined organic phases were washed with brine (2 x 20ml), dried ($MgSO_4$) and evaporated. The residue was chromatographed on silica using 2:1 petrol:ethyl acetate as eluent, to afford the title compound as a colourless solid (320mg, 56%). ^1H NMR (250MHz, D_6-DMSO) δ 0.63 (3H, d, J = 7Hz), 0.77 (3H, d, J = 7Hz), 1.26 (9H, s), 1.49 (1H, m), 3.56 (1H, dd, J = 4Hz, 14Hz), 4.10 (1H, dd, J = 4Hz, 14Hz), 4.88 (1H, m), 5.02 (2H, m), 7.20-7.40 (5H, m), 7.58 (1H, t, J = 8Hz), 7.66 (1H, d, J = 9Hz), 7.77 (1H, d, J = 8Hz), 8.18 (1H, d, J = 9Hz).

Step 6: 1,3-Dihydro-5-(1,1-dimethylethyl)-1-(2-methylpropyl)-3(R,S)-[(4-nitrophenyloxycarbonyl)amino]-2H-1,4-benzodiazepin-2-one

1,3-Dihydro-5-(1,1-dimethylethyl)-1-(2-methylpropyl)-3(R,S)-[(benzyloxycarbonyl)amino]-2H-1,4-benzodiazepin-2-one (400mg, 0.95mmol) was dissolved in formic acid/methanol (13ml of a 4.5% (v/v) solution), and added to a stirred suspension of 10% palladium on carbon (50mg, 12.5% ($^{w}/_{w}$)) in formic acid/methanol (12ml of a 4.5% (v/v) solution)). After 30 min the catalyst was removed by filtration, the filtrate evaporated and the residue partitioned between 10% sodium carbonate solution (20ml) and dichloromethane (3 x 20ml). The combined organic phase was dried (Na_2SO_4) and evaporated to give a colourless solid, which was used without further purification.

A solution of the crude 3(R,S)-amino-1,3-dihydro-5-(1,1-dimethylethyl)-1-(2-methylpropyl)-2H-1,4-benzodiazepin-2-one (200mg, 0.7mmol) in dry THF (15ml) under an atmosphere of nitrogen at 0°C was treated with triethylamine (0.10ml, 0.7mmol) followed by a solution of 4-nitrophenylchloroformate (141mg, 0.7mmol) in THF (15ml). After stirring at room temperature for 20 min the solid which precipitated was removed by filtration and the filtrate concentrated *in vacuo* to afford 160mg (51% yield) of the titled compound as a colourless solid. ^{1}H NMR (360MHz, $CDCl_3$) δ 0.76 (3H, d, J = 7Hz), 0.84 (3H, d, J = 7Hz), 1.34 (9H, s), 1.69 (1H, m), 4.31 (1H, m), 5.11

(1H, d, J = 8Hz), 6.64 (1H, d, J = 8Hz), 7.20-7.60 (5H, m), 7.70 (1H, m), 8.22 (2H, m).

Step 7: N[3(R,S)-2,3-Dihydro-5-(1,1-dimethylethyl)-1-(2-methylpropyl)-2-oxo-1H-1,4-benzodiazepin-3-yl] N'-[3-(methylsulphonylaminocarbonyl)phenyl]urea

A solution of -1,3-dihydro-5-(1,1-dimethylethyl)-1-(2-methylpropyl)-3(R,S)-[(4-nitrophenyloxycarbonyl)amino]-2H-1,4-benzodiazepin-2-one (140mg, 0.3mmol) in anhydrous dimethylformamide (3ml) under an atmosphere of nitrogen was treated with triethylamine (0.043ml, 0.3mmol) and stirred at ambient temperature for 5 min. To the reaction mixture was added dropwise a solution of 1-(methylsulphonylaminocarbonyl)-3-aminobenzene (66mg, 0.3mmol) in anhydrous dimethylformamide (3ml). The reaction was stirred at 50°C for 3.5 h. The solvent was evaporated *in vacuo* and the residue partitioned between ethyl acetate (15ml) and aqueous acetic acid (20%, 5ml). The layers were separated and the aqueous phase washed with ethyl acetate (2 x 15ml). The combined organics were dried ($MgSO_4$), evaporated and triturated with ether to give a beige solid. The crude product was recrystallised from methanol to afford the title compound as a colourless solid (60mg, 38%) mp = 250°C (dec.). ^1H NMR (360MHz, D_6-DMSO) δ 0.65 (3H, d, J = 7Hz), 0.79 (3H, d, J = 7Hz), 1.26 (9H, s), 1.51 (1H, m), $MeSO_2$ peak, 3.35 (3H, s), 3.59 (1H, m), 4.13 (1H, m), 5.03 (1H, m), 7.30-8.00 (9H, m), 9.16 (1H, s), 12.08 (1H, s).

EXAMPLE 4A Tablets containing 1-25mg of compound

	Amount mg		
Compound of formula (I)	1.0	2.0	25.0
Microcrystalline cellulose	20.0	20.0	20.0
Modified food corn starch	20.0	20.0	20.0
Lactose	58.5	57.5	34.5
Magnesium Stearate	0.5	0.5	0.5

EXAMPLE 4B Tablets containing 26-100mg of compound

	Amount mg		
Compound of formula (I)	26.0	50.0	100.0
Microcrystalline cellulose	80.0	80.0	80.0
Modified food corn starch	80.0	80.0	80.0
Lactose	213.5	189.5	139.5
Magnesium Stearate	0.5	0.5	0.5

The compound of formula (I), cellulose, lactose and a portion of the corn starch are mixed and granulated with 10% corn starch paste. The resulting granulation is sieved, dried and blended with the remainder of the corn starch and the magnesium stearate. The resulting granulation is then compressed into tablets containing 1.0mg, 2.0mg, 25.0mg, 26.0mg, 50.0mg and 100mg of the active compound per tablet.

EXAMPLE 5 Parenteral injection

	Amount mg
Compound of formula (I)	1 to 100
Citric Acid Monohydrate	0.75
Sodium Phosphate	4.5
Sodium Chloride	9
Water for Injections	to 1ml

The sodium phosphate, citric acid monohydrate and sodium chloride are dissolved in a portion of the water. The

compound of formula (I) is dissolved or suspended in the solution and made up to volume.

EXAMPLE 6 Topical formulation

	Amount mg
Compound of formula (I)	1-10
Emulsifying Wax	30
Liquid paraffin	20
White Soft Paraffin	to 100

The white soft paraffin is heated until molten. The liquid paraffin and emulsifying wax are incorporated and stirred until dissolved. The compound of formula (I) is added and stirring continued until dispersed. The mixture is then cooled until solid.

BIOLOGICAL ACTIVITY

1. CCK Receptor Binding (Pancreas)

CCK-8 sulphated was radiolabelled with ^{125}I-Bolton Hunter reagent (2000 Ci/mmole). Receptor binding was performed according to Chang and Lotti (Proc. Natl. Acad. Sci. 83, 4923-4926, 1986) with minor modifications.

Male Sprague-Dawley rats (150-200g) were sacrificed by decapitation. The whole pancreas was dissected free of fat tissue and was homogenized in 25 volumes of ice-cold 10 mM N-2-hydroxyethyl-piperazine-N'-2-ethane sulphonic acid (HEPES) buffer with 0.1% soya bean trypsin inhibitor (pH 7.4 at 25°C) with a Kinematica Polytron. The homogenates were centrifuged at 47,800 g for 10 min. Pellets were resuspended in 10 volumes of binding assay buffer (20mM (HEPES)), 1mM ethylene glycol-bis-(β-aminoethylether-N,N'-tetraacetic acid) (EGTA), 5mM $MgCl_2$, 150 mM NaCl, bacitracin 0.25 mg/ml, soya bean trypsin inhibitor 0.1 mg/ml, and bovine serum albumin 2

mg/ml pH 6.5 at 25°C) using a Teflon (trademark) homogenizer, 15 strokes at 500 rpm. The homogenate was further diluted in binding assay buffer to give a final concentration of 0.5 mg original wet weight/1 ml buffer. For the binding assay, 50 µl of buffer (for total binding) or unlabelled CCK-8 sulphated to give a final concentration of 1 µM (for nonspecific binding) or the compounds of Formula I (for determination of inhibition of ^{125}I-CCK-8 binding) and 50 µl of 500 pM ^{125}I-CCK-8 (i.e. 50 pM final concentration) were added to 400 µl of the membrane suspensions in microfuge tubes. All assays were run in duplicate. The reaction mixtures were incubated at 25°C for 2 hours and the reaction terminated by rapid filtration (Brandell 24 well cell harvester) over Whatman GF/C filters, washing 3 x 4 mls with ice-cold 100 Mm NaCl. The radioactivity on the filters was counted with a LKB gamma counter.

2. <u>CCK Receptor Binding (Brain)</u>

CCK-8 sulphated was radiolabelled and the binding was performed according to the description for the pancreas method with minor modifications.

Male Hartley guinea pigs (300-500g) were sacrificed by decapitation and the cortex was removed and homogenized in 25 mL ice-cold 0.32 M sucrose. The homogenates were centrifuged at 1000 g for 10 minutes and the resulting supernatant was recentrifuged at 20,000 g for 20 minutes. The P_2 pellet was resuspended in binding assay buffer (20mM HEPES, 5 mM $MgCl_2$, 0.25 mg/ml bacitracin, 1 mM EGTA pH 6.5 at 25°C), using a Teflon (trademark) homogenizer (5 strokes at 500 rpm) to give a final concentration of 10 mg original wet weight/1.2 ml buffer. For the binding assay, 50 µl of buffer (for total binding) or unlabelled CCK-8 sulphated to give a

final concentration of 1 μM (for nonspecific binding) or the compounds of Formula I (for determination of inhibition of ^{125}I-CCK-8 binding) and 50 μl of 500 pM ^{125}I-CCK-8 (i.e. final concentration of 50 pM) were added to 400 μl of the membrane suspensions in microfuge tubes. All assays were run in duplicate. The reaction mixtures were incubated at 25°C for 2 hours and then the reaction was terminated by rapid filtration (Brandell 24 well cell harvester) on Whatman GF/C filters with 3 x 5 ml washes of cold 100 mM NaCl. The radioactivity on the filters was counted with a LKB gamma counter.

In Vitro Results

Effects of the Compounds of Formula I on ^{125}I-CCK-8 receptor binding

The preferred compounds of Formula I are those which produced dose-dependent inhibition of specific ^{125}I-CCK-8 binding as defined as the difference between total and non-specific (i.e. in the presence of 1 μM CCK) binding.

Drug displacement studies were performed with at least 10 concentrations of compounds of Formula I and the IC_{50} values were determined by regression analysis IC_{50} refers to the concentration of the compound required to inhibit 50% of specific binding of ^{125}I-CCK-8.

The data in Table I were obtained for compounds of Formula I.

TABLE I

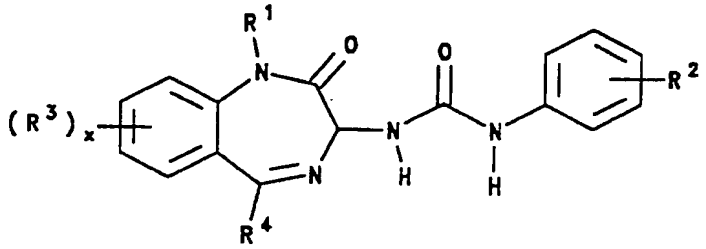
CCK RECEPTOR BINDING RESULTS

IC_{50}(nM)

Compound of Ex #	^{125}I-CCK Pancreas	^{125}I-CCK Brain
1	>3000	104
2	600	17
3	612	32

CLAIMS:

1. A compound of formula (I):



(I)

wherein:

R^1 represents H, C_{1-6}alkyl, C_{3-7}cycloalkyl, cyclopropylmethyl, $CH_2CO_2R^5$ (where R^5 is C_{1-4}alkyl) or a group $CH_2CONR^6R^7$ (where R^6 and R^7 each independently represents H or C_{1-4}alkyl, or R^6 and R^7 together form a chain $(CH_2)_p$ where p is 4 or 5);

R^2 represents $(CH_2)_q$-tetrazolyl optionally substituted in the tetrazole ring by C_{1-4}alkyl, $(CH_2)_q$-imidazolyl (where q is 0, 1, 2 or 3), $CONHSO_2R^9$, SO_2NHCOR^9 (where R^9 is C_{1-6}alkyl, optionally substituted aryl or trifluoromethyl), SO_2NHR^{10} (where R^{10} is a nitrogen containing heterocycle), cyclopropyl or $(CH_2)_nCO_2H$, where n is 1 or 2;

R^3 represents C_{1-6}alkyl, halo or $NR^{16}R^{17}$, where R^{16} and R^{17} each independently represent H or C_{1-4}alkyl, or R^{16} and R^{17} together form a chain $(CH_2)_r$ where r is 4 or 5;

R^4 represents C_{1-7} straight or branched chain alkyl;

x is 0, 1, 2 or 3;

or a salt or prodrug thereof.

2. A compound as claimed in claim 1 wherein R^2 represents $CONHSO_2R^9$ or SO_2NHCOR^9.

3. A compound as claimed in claim 1 wherein R^2 represents tetrazolyl.

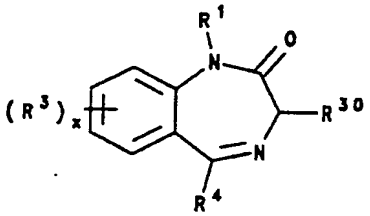
4. A compound as claimed in any preceding claim wherein R^1 represents C_{1-6}alkyl.

5. A compound as claimed in claim 1 selected from:
N-[3(R,S)-2,3-dihydro-1-(2-methylpropyl)-2-oxo-5-(2-propyl)-1H-1,4-benzodiazepin-3-yl] N'-[3-tetrazol-5-ylphenyl]urea;
N-[3(R,S)-2,3-dihydro-1,5-dimethyl-2-oxo-1H-1,4-benzodiazepin-3-yl] N'-[3-tetrazol-5-ylphenyl]urea;
N-[3(R,S)-2,3-dihydro-5-(1,1-dimethylethyl)-1-(2-methylpropyl)-2-oxo-1H-1,4-benzodiazepin-3-yl] N'-[3-(methylsulphonylaminocarbonyl)phenylurea;
and salts and prodrugs thereof.

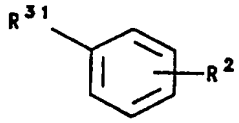
6. A compound as claimed in any preceding claim for use in therapy.

7. A pharmaceutical composition comprising a compound as claimed in any of claims 1 to 5 in association with a pharmaceutically acceptable carrier or excipient.

8. A process for the preparation of a compound as claimed in any of claims 1 to 5, which process comprises reacting a compound of formula (II) with a compound of formula (III):



(II)



(III)

wherein R^1, R^2, R^3, R^4 and x are as defined for formula (I), one of R^{30} and R^{31} represents NH_2 and the other of R^{30} and R^{31} represents N=C=O or an activated carbamate; and optionally converting the compound of formula (I) so prepared to a salt or prodrug thereof.

9. The use of a compound as claimed in any of claims 1 to 5 for the manufacture of a medicament for the treatment of a physiological disorder involving CCK and/or gastrin.

10. The use of a compound as claimed in any of claims 1 to 5 for the manufacture of a medicament for the treatment of panic, anxiety or pain.

11. A compound as claimed in any of claims 1 to 5 when prepared by the process of claim 8.

12. A process for preparing a composition as claimed in claim 7 which process comprises bringing a compound as claimed in any of claims 1 to 5 into

association with a pharmaceutically acceptable carrier or excipient.

13. A compound, composition or process as claimed in any one of the preceding claims, substantially as hereinbefore described.

14. A method for the treatment or prevention of a physiological disorder involving CCK and/or gastrin, which method comprises administration to a patient in need thereof of a CCK and/or gastrin reducing amount of a compound according to claim 1.

15. A method as claimed in claim 14 for the treatment or prevention of anxiety.

16. A method as claimed in claim 14 for the treatment or prevention of panic.

17. A method as claimed in claim 14 for the treatment of pain.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 93/00299

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C07D403/12; C07D243/14; A61K31/55; C07D521/00

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07D ; A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	EP,A,0 434 364 (MERCK) 26 June 1991 see claims ---	1,7-13
P,A	EP,A,0 514 133 (MERCK) 19 November 1992 see claims -----	1,6-13

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
17 MAY 1993	25. 05. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	FRANCOIS J.C.

INTERNATIONAL SEARCH REPORT

ernational application No.
PCT/GB93/00299

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 14-17 are directed to a method of treatment of the human body, the search has been carried out and based on the attributed effects of the compounds.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

GB 9300299
SA 70196

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 17/05/93

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0434364	26-06-91	AU-A- 6815190	20-06-91
EP-A-0514133	19-11-92	None	